ELSEVIER

Journal of Cystic Fibrosis 7 (2008) 555–565

Journal of Cystic Fibrosis

www.elsevier.com/locate/jcf

# Anti-inflammatory effect of miglustat in bronchial epithelial cells☆

Maria Cristina Dechecchi [a,*], Elena Nicolis [a], Caroline Norez [a,b], Valentino Bezzerri [a], Monica Borgatti [c], Irene Mancini [c], Paolo Rizzotti [a], Carla M.P. Ribeiro [d], Roberto Gambari [c], Frederic Becq [b], Giulio Cabrini [a]

[a] *Laboratory of Molecular Pathology, Laboratory of Clinical Chemistry and Haematology, University Hospital of Verona, Verona, Italy*
[b] *Institut de Physiologie et Biologie Cellulaires, Université de Poitiers, CNRS, Poitiers, France*
[c] *Department of Biochemistry and Molecular Biology, University of Ferrara, Italy*
[d] *Department of Medicine and Cystic Fibrosis Pulmonary Research and Treatment Center, University of North Carolina, Chapel Hill, NC, USA*

Received 4 March 2008; received in revised form 6 June 2008; accepted 26 June 2008
Available online 23 September 2008

**Abstract**

The role of CFTR deficiency in promoting inflammation remains unclear. Perez et al. [A. Perez, A.C. Issler, C.U. Cotton, T.J. Kelley, A.S. Verkman and P.B. Davis, CFTR inhibition mimics the cystic fibrosis inflammatory profile. Am J Physiol Lung Cell Mol Physiol 2007; 292:L383–L395.] recently demonstrated that the inhibition of function of w/t CFTR produces an inflammatory profile that resembles that observed in CF patients, whereas we found that correction of F508del-CFTR function with MPB-07 down-modulates the inflammatory response to *P. aeruginosa* in CF bronchial cells [M.C. Dechecchi, E. Nicolis, V. Bezzerri, A. Vella, M. Colombatti, B.M. Assael, et al., MPB-07 reduces the inflammatory response to Pseudomonas aeruginosa in cystic fibrosis bronchial cells. Am J Respir Cell Mol Biol 2007; 36, 615–624.]. Since both evidence support a link between CFTR function and inflammation, we extended our investigation to other F508del-CFTR correctors, such as miglustat (Norez, 2006), an approved drug for Gaucher disease, in comparison with the galactose analogue NB-DGJ. We report here that miglustat but not NB-DGJ restores F508del-CFTR function in CF bronchial epithelial IB3-1 and CuFi-1 cells. Miglustat and NB-DGJ reduce the inflammatory response to *P. aeruginosa* in both CF and non-CF bronchial cells, indicating that the anti-inflammatory effect is independent of the correction of F508del-CFTR function. Miglustat also inhibits the inflammatory response induced by the supernatant of mucopurulent material obtained from the lower airway tract of cystic fibrosis patients with chronic bacterial colonization (Ribeiro, 2005). Both compounds do not interfere with the adherence of *P. aeruginosa* to the cells and reduce the expression of IL-8 not only after challenge with *P. aeruginosa* but also after exposure to TNF alpha or IL-1 beta, suggesting an effect on transduction proteins downstream and in common with different receptors for pathogens. Finally, miglustat has no major effects on overall binding activity of transcription factors NF-κBNF-*k*B and AP-1. Since miglustat is an approved drug, it could be investigated as a novel anti-inflammatory molecule to ameliorate lung inflammation in CF patients.


*Keywords:* Cystic fibrosis; Information response; pseudomonas aeruginosa; CFTR function; correctors

## 1. Background

Airway epithelial cells have their own repertoire of innate immune functions. Once pathogens invade the airways, the epithelium expresses chemokines and cytokines to recruit and activate neutrophils, thus contributing to initiate the overall inflammatory response in the lung. Airway inflammation is a hallmark of cystic fibrosis (CF) lung disease that is characterized by persistent bacterial infection, prevalently by *Pseudomonas aeruginosa* (*P. aeruginosa*), increased number of neutrophils and elevated levels of cytokines in the airway

☆ Data from this manuscript have been presented at: 21st Annual North American Cystic Fibrosis Conference, Anaheim California, 3-6 October, 2007 and XII Italian Cystic Fibrosis Congress-III Congress SIFC, Milan, Italy, 30th November–2nd December 2007.

* Corresponding author. Laboratory of Molecular Pathology-Laboratory of Clinical Chemistry and Haematology, University Hospital of Verona, Verona, Italy, Azienda Ospedaliera di Verona-Piazzale Stefani 1, 37126, Verona, Italy. Tel.: +39 0458122191; fax: +39 0458122840.
*E-mail address:* cristina.dechecchi@azosp.vr.it (M.C. Dechecchi).

1569-1993/$ - see front matter © 2008 European Cystic Fibrosis Society. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.jcf.2008.06.002

fluids [1–3]. There is a consensus that in CF the inflammatory response to infection is dysregulated and excessive, however it remains enigmatic how abnormalities in the cystic fibrosis transmembrane conductance regulator (CFTR) can be responsible for the exuberant and persistent pulmonary infection and inflammation. The most prominent hypotheses propose that defective CFTR causes a decrease in the airway surface liquid (ASL) which makes more difficult to efficiently clear infected secretions from the lung [4], leading to a chronic obstruction and bacterial infection with Gram-negative organisms growing in biofilms, especially *P. aeruginosa*, thus triggering a dramatic inflammatory response. It has been controversial whether this hyperinflammatory milieu is only the result of the chronic infection or it is primary to the CFTR defect [5]. This controversy is difficult to resolve since most of the studies have been performed comparing cell cultures developed from normal and CF individuals which differ at many genetic loci besides the CF gene, thus increasing the variability and making unlikely to ascribe responses only to defective CFTR. Recently, the CFTR-specific inhibitor $CFTR_{inh}$-172 was used by Perez et al., to create a CF model with its own control to test whether the absence of CFTR-dependent Cl-conductance by itself was sufficient to produce inflammatory changes observed in matched cell lines [7]. They demonstrated that the inhibition of function of CFTR mimics the CF inflammatory profile, supporting the hypothesis that lack of CFTR activity accounts for the onset of the inflammatory cascade in the CF lung. On the other hand, we have previously shown that in CF bronchial cells, the correction of F508del-CFTR function with the corrector benzo(*c*)quinolizinium (MPB)-07 strongly reduces the inflammatory response induced by *P. aeruginosa* [8]. These complementary lines of evidence suggest that the pro-inflammatory circuitry in CF airways could be initiated from those surface epithelial cells lacking CFTR function. Therefore, in the present work we have further explored the possibility to control the inflammatory response to *P. aeruginosa* in CF bronchial cells through the pharmacological modulation of CFTR defect. These studies are in line with the concept that knowledge of the pharmacotherapy for defective CFTR is on an exponential increase as high-throughput screening of chemically different compounds is revealing a great number of activators [6,9,10] and correctors of defective processing [10–12].

In this respect, glycosidases are involved in the biosynthesis of the oligosaccharide chains and quality control mechanisms of the *N*-linked glycoproteins and glycolipids and have been implicated in the development of various diseases including viral infection, cancer and genetic disorders [13]. The iminosugar *N*-butyldeoxynojirimycin (miglustat), an inhibitor of glycolipid biosynthesis [14] as been proposed for treating type I Gaucher disease, an inherited glycosphingolipid (GSL) lysosomal storage disease [15]. In addition to the inhibitory effect on the glycolipid biosynthesis, miglustat is a potent α-glucosidase inhibitor and may also works as a pharmaceutical chaperone [16].

We have recently demonstrated that miglustat restores functional F508del-CFTR channels in human and mice epithelial CF cells [17]. There is no information regarding the effects of miglustat on the inflammatory response in CF epithelial cells.

In the present study, airway inflammatory response to *P. aeruginosa* was investigated by measuring the expression of interleukin (IL)-8 and intercellular adhesion molecule-1 (ICAM-1) induced by the *P. aeruginosa* laboratory strain PAO1 in CF bronchial cells treated with miglustat, in comparison with the galactose analogue *N*-butyldeoxygalactonojirimycin (NB-DGJ). The effect of miglustat was also studied in CF cells stimulated by pooled supernatants of mucopurulent material (SMM) collected from the lower airway tract of the lungs of CF patients with chronic bacterial colonization obtained at the time of lung transplantation [18–19]. In addition to IB3-1 and CuFi-1, the possible anti-inflammatory effect of miglustat was analyzed in non-CF NuLi-1 cells. The effects of miglustat on the expression of IL-8 and ICAM-1 were compared with the analysis of the activity of two transcription factors (NF-κB and AP-1).

Since miglustat is an approved drug, it could represent an interesting new molecule to ameliorate lung inflammation in CF patients. Therefore, analysis of its effects on bronchial CF and non-CF cells is mandatory to reach a general commitment about the use of miglustat in novel therapeutic approaches based on ability to restore functional CFTR channels and to reduce inflammation.

## 2. Methods

### 2.1. Cell lines and bacteria

IB3-1 is a human bronchial epithelial cell line, immortalised with adeno12/SV40, derived from a CF patient with a F508del/W1282X mutant genotype [20]. These cells have been obtained from LGC Promochem, Europe and were grown in LHC-8 basal medium (Biofluids Inc., Rockville, MO) supplemented with 5% FBS. All culture flasks and plates were coated with a solution containing 35 μg/ml bovine collagen (Becton–Dickinson, Franklin Lakes, NJ), 1 μg/ml BSA (Sigma, St. Louis, MO) and 1 μg/ml human fibronectin (Becton–Dickinson) as described [20]. CuFi-1 and NuLi-1 cells, a generous gift of A. Klingelhutz, P. Karp and J. Zabner (University of Iowa, Iowa City) derived from human bronchial epithelium from a CF patient (CuFi-1, F508del/F508del CFTR mutant genotype) or a non-CF subject (NuLi-1, wild type CFTR) and have been transformed by the reverse transcriptase component of telomerase, hTERT, and human papillomavirus type 16 (HPV-16) E6 and E7 genes [21]. These cell lines were grown on human placental collagen type VI (Sigma, St. Louis, MO) coated flasks in BEGM medium (Cambrex Bio Science Walkersville, MD), as described [20]. PAO1, a prototypic laboratory strain of *P. aeruginosa*, kindly provided by A. Prince (Columbia University, NewYork) was grown in trypticase soy broth (TSB) or agar (TSA) (Difco, Detroit MI).

### 2.2. CFTR function assay

#### 2.2.1. Single-cell fluorescence imaging

IB3-1 and CuFi-1 cells were incubated with 200 μM miglustat or NB-DGJ (Toronto Research Chemicals, North

York, ON, Canada) for 2, 4 and 24 h. CFTR function was assessed by single-cell fluorescence imaging, using the potential-sensitive probe DiSBAC2[3] (Molecular Probes, Eugene, OR) as previously reported [8]. CFTR-dependent Cl-channel was stimulated by a cAMP elevating cocktail: 20 μM forskolin plus 100 μM IBMX and 50 μM genistein. The thiazolidinone CFTR inhibitor CFTRinh-172, kindly provided by A. S.Verkman (University of California, San Francisco) [22], was added to a final concentration of 10 μM.

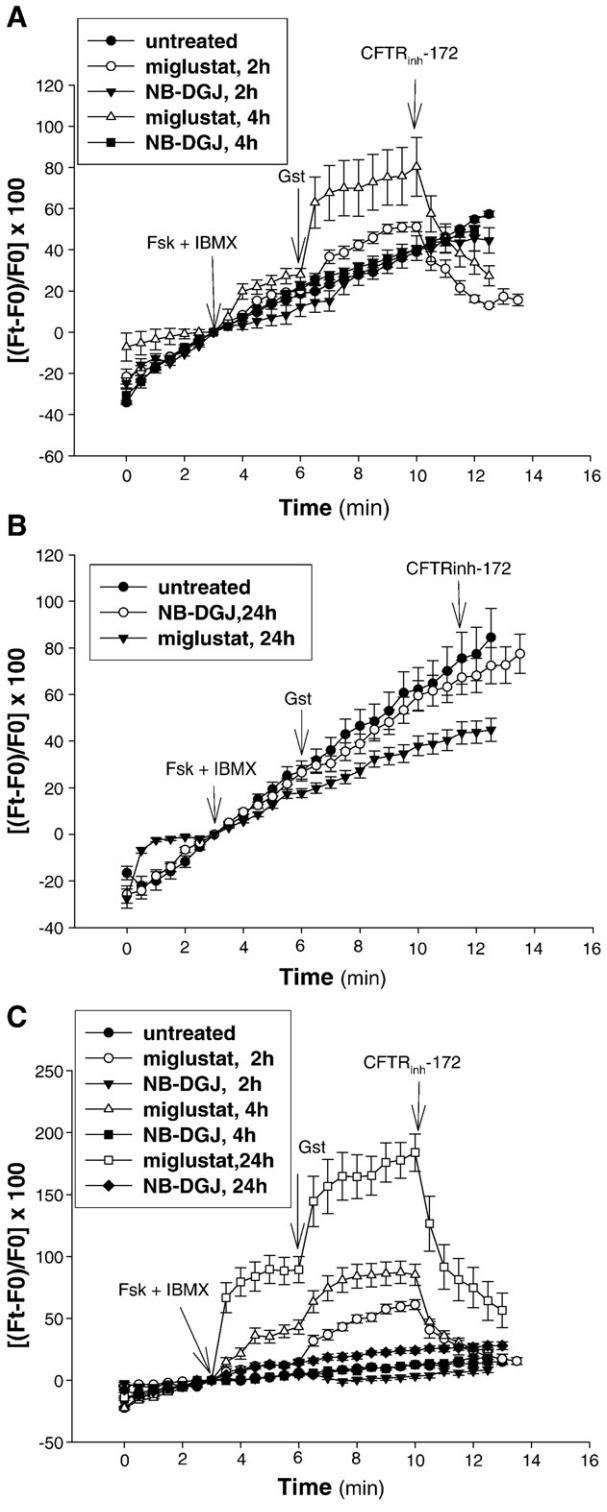


Fig. 1. Miglustat restores F508del-CFTR function in IB3-1 and CuFi-1 bronchial cells. IB3-1 cells grown on round glass coverslips were incubated with miglustat or NB-DGJ (200 μM) for 2 and 4 h (A) or 24 h (B) and then mounted on the perfusion chamber and perfused with Cl-free solution containing DiSBAC2 [3] to allow the equilibration of the dye within cell membranes. The arrows indicate the time of the addition of the stimuli or the inhibitor. Fluorescence coming from each single cell was analyzed. Typical time courses are shown. Data represents the mean±SEM of the relative fluorescence collected from all the cells of the field ($n$=9 A, $n$=11 B). Representative of 6 (A) or 4 (B) independent experiments are shown C. CuFi-1 cells grown on round glass coverslips were incubated with miglustat or NB-DGJ as indicated for A and B and then mounted on the perfusion chamber and the experiment performed as indicated above. Data represents the mean±SEM of the relative fluorescence collected from all the cells of the field ($n$=9). Representative of 6 independent experiments is shown.

### 2.2.2. Iodide efflux

CuFi-1 cells were grown in multiwell plates and incubated with miglustat ranging from 1 nM to 100 μM for 24 h. Iodide efflux was performed as described [23]. Cells cultured in multiwell plates were washed twice with efflux buffer containing (in mM) 136.9 NaCl, 5.4 KCl, 0.3 $KH_2PO_4$, 0.3 $NaH_2PO_4$, 1.3 $CaCl_2$, 0.5 $MgCl_2$, 0.4 $MgSO_4$, 5.6 glucose and 10 HEPES, pH 7.4 and incubated in efflux buffer containing $Na^{125}I$ (1 μCi $Na^{125}I$/ml, NEN, Boston, MA) during 1 h at 37 °C. Cells were then washed with efflux medium to remove extracellular $^{125}I$. The loss of intracellular $^{125}I$ was determined by removing the medium with efflux buffer every 1 min for up to 10 min. The first three aliquots were used to establish a stable baseline in efflux buffer alone. A medium containing the appropriate drug was used for the remaining aliquots. Residual radioactivity was extracted with 0.1 N NaOH/0.1% SDS. The fraction of initial intracellular $^{125}I$ lost during each time point was collected and time-dependent rates of $^{125}I$ efflux calculated from: $\ln(I_{t1}\ {}^{125}I_{t1}/{}^{125}I_{t2})/(t_1-t_2)$ where $^{125}I_t$ is the intracellular $^{125}I$ at time $t$, and $t_1$ and $t_2$ successive time points. Curves were constructed by plotting rate of $^{125}I$ versus time. All comparisons were based on maximal values for the time-dependent rates ($k$=peak rates, $min^{-1}$) excluding the points used to establish the baseline ($k$ peak–$k$ basal, $min^{-1}$). The rate of activation was determined after mathematical fitting. The $EC_{50}$ was calculated using the software GraphPad Prism version 4.0 for Windows (Graphpad Software).

## 2.3. Inflammatory response

Cells were seeded at density ranging from 50,000 to 100,000 cells/$cm^2$. After adhesion, IB3-1 cells were starved in serum free LHC-8 for 18 h. Before the experiment, bacteria from overnight cultures on TSA plates were grown in 20 ml TSB broth at 37 °C with shaking until there was an OD at 660 nm of about $1\times10^9$ CFU/ml, determined by dilution plating. Monolayers of cells were treated or not with 200 μM miglustat or NB-DGJ, dissolved in water, for 24 h and then infected with PAO1 as described [8], or stimulated by the pro-inflammatory cytokines TNF alpha [10 ng/ml] or IL-1 beta [50 ng/ml] (Human recombinant, Sigma, St. Louis, MO), for 4 h at 37 °C. Monolayer of cells were incubated for 4 h with supernatants of mucopurulent material (SMM) pooled from samples collected

from the lower airways of 16 CF patients infected with *P. aeruginosa* and *Staphylococcus aureus*, obtained as described [18–19], from lungs at the time of transplantation, diluted 1:25 in medium. The expression of mRNA of IL-8 and ICAM-1 was measured by Real-time qPCR as described [8].

### 2.4. Electrophoretic mobility shift assay (EMSA)

Cells were seeded on coated Petri dishes (5 cm diameter) at density of 100,000/cm$^2$ 24 h before infection, infected as described above for 2 h and detached by trypsin. DNA-binding proteins were extracted by hypotonic lysis followed by high salt extraction of nuclei [24]. EMSA was perfomed as previously described [25]. Briefly, double-stranded synthetic oligodeoxynucleotides mimicking the NF-κB and the AP-1 binding sites (NF-κB, sense: 5′-AATCGTGGAATTTCCTCT-3′, AP-1 sense: 5′-TGTGATGACTCAGGTTTG-3′) have been employed. Oligodeoxynucleotides were labeled with $\gamma^{32}$-P-ATP using 10 Units of T4-polinucleotide-kinase (MBI Fermentas) in 500 mM Tris–HCl, pH 7.6, 100 mM $MgCl_2$, 50 mM DTT, 1 mM spermidine, 1 mM EDTA in the presence of 50 μCi $\gamma^{32}$-P-ATP) in a volume of 20 μl for 45 min at 37 °C. Reaction was brought to 150 mM NaCl and 150 ng complementary oligodeoxynucleotide was added. Reaction temperature was increased to 100 °C for 5 min and left diminishing to room temperature overnight. Nuclear extracts from cells were used at concentrations ranging from 0.5 to 2 μg/reaction concentrations in the presence of poly (dI:dC) (1 mg/reaction) to abolish non-specific binding. After 25 min binding at room temperature, the samples were run at constant voltage (200 V) under low ionic strength conditions (0.25× TBE buffer: 22 mM Tris-borate, 0.4 mM EDTA) on 6% polyacrylamide gels. Gels were dried and subjected to standard autoradiographic procedures.

### 2.5. Adherence of PAO1 to IB3-1 and CuFi-1 cells

PAO1 were metabolically labelled with [35S] methionine according to [26] with minor changes. Colonies of PAO1 from overnight TSA plates were inoculated in 20 ml M9 medium and grown at 37 °C with shaking to a density of 108 CFU/ml. M9 medium (Difco, Detroit MI) was used to have high-specific activity labelling. 100 μCi/ml [35 S] methionine (Amersham Biosciences, Uppsala, Sweden) was added to the broth and incubated at 37 °C with shaking for 30 min. Bacteria were then washed twice with 10 mM NaCl and resuspended in PBS. Aliquots of the bacterial suspension were plated and scintillations counted to calculate the number of bacteria associated with the counts per minute (CFU/cpm). Specific activity ranged between 40 and 1000 CFU/cpm.

Metabolically labelled PAO1 were added to monolayers of IB3-1 or CuFi-1 cells, in duplicate, and incubated at room temperature for 60 min. Unbound organisms were rinsed off the monolayers with three successive PBS washes. Cells and adherent bacteria were solubilized in 0.5 ml of 2% SDS and scintillations were counted. Specific binding was calculated by subtracting counts obtained in the presence of 100-fold excess unlabelled PAO1. Non-specific binding was about 30% of total.

### 2.6. Statistics

Results are expressed as mean ± standard error of the mean. Comparisons between groups were made by using Student's *t* test and a one-way analysis of variance (ANOVA). Statistical significance was defined with $p < 0.05$.

## 3. Results

### 3.1. Functional evaluation of CFTR in airway epithelial cells treated with miglustat

We have previously demonstrated that the treatment for 2 h with miglustat restores the CFTR-dependent Cl-transport in human nasal epithelial JME/CF15, tracheal gland serous CF-KM4, pancreatic duct CFPAC-1 cell lines and intestinal cells of F508del-CFTR mice [17]. Therefore CFTR function was tested here in bronchial IB3-1 and CuFi-1 cell lines treated with miglustat or with NB-DGJ. IB3-1 cells were incubated for 2, 4 and 24 h in the presence of 200 μM miglustat or NB-DGJ. As shown in Fig. 1, the fluorescence signal does not change in untreated cells or in cells treated with NB-DGJ, whereas the addition of the cAMP stimulation cocktail plus genistein increases the fluorescence after 2 and, more evidently, 4 h treatment with miglustat. The sharp decrease observed after the addition of $CFTR_{inh}$-172 indicates the CFTR-specific functional

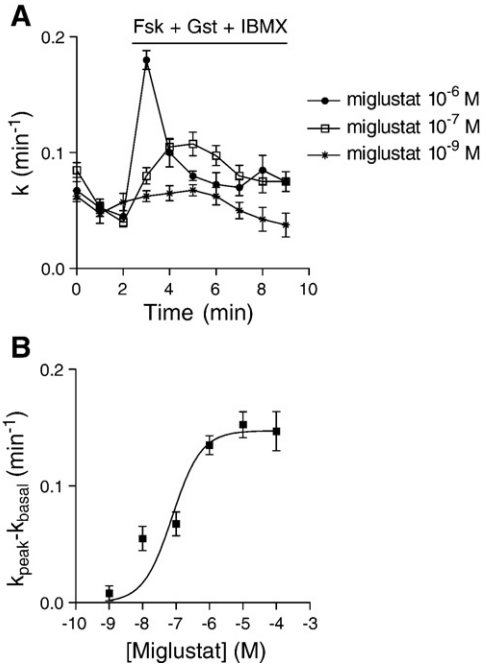


Fig. 2. Dose–response of rescue of F508del-CFTR function by miglustat in CuFi-1 cells. Cells seeded on multiwell plates were treated for 24 h with various concentration of miglustat. A. Example of mean traces showing the restoration of an iodide efflux on CuFi-1 cells treated 24 h with various concentration of miglustat and stimulated by a cAMP cocktail (forskolin 20 μM+IBMX 100 μM)+genistein 50 μM. B. Concentration–response curve showing CFTR-dependent iodide efflux at each miglustat concentration. $n=4$ for each concentration.

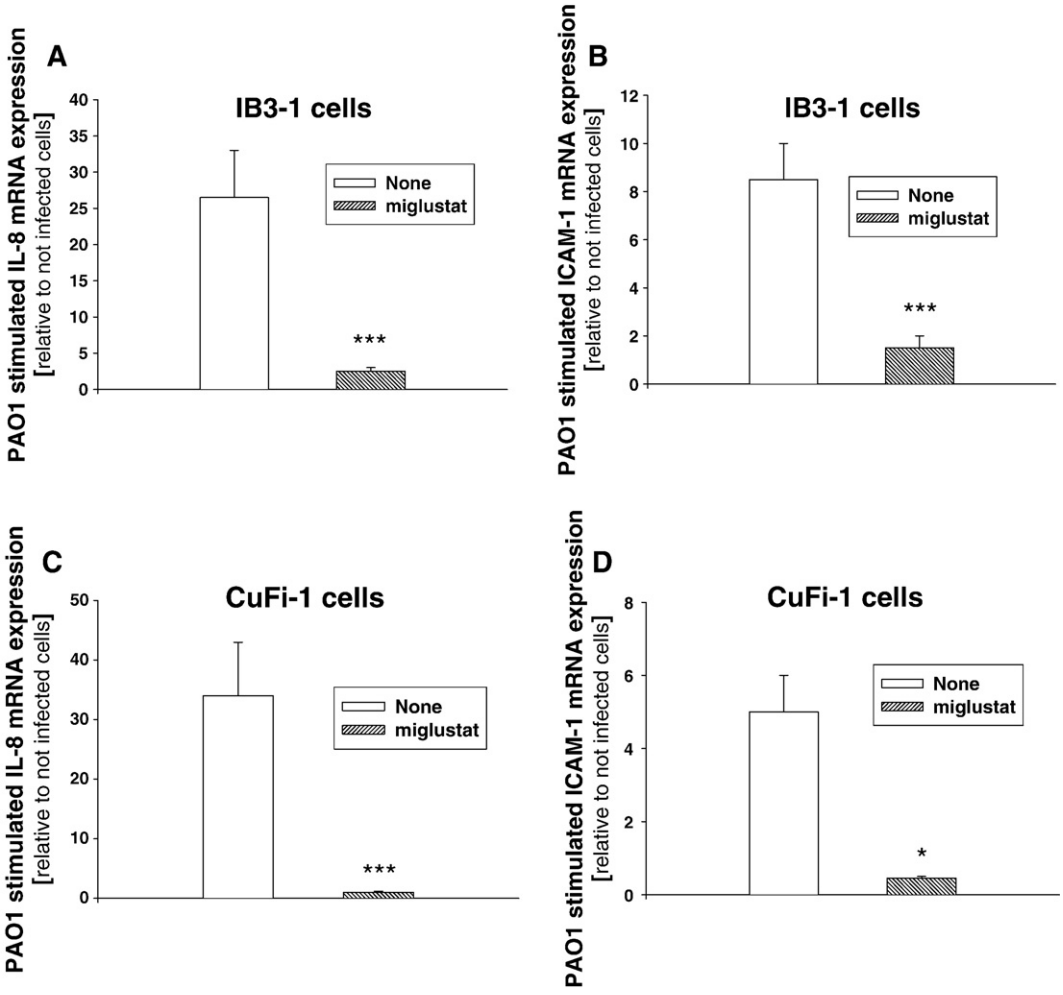


Fig. 3. Miglustat reduces the expression of mRNA of IL-8 and ICAM-1, stimulated by PAO1 in IB3-1 and CuFi-1 cells. Cells were incubated for 24 h in the presence of 200 μM miglustat and infected with PAO1 (50 CFU/cell) for 4 h as described in the Materials and methods section. The mRNA induction (relative to non-infected cells) is obtained by comparing the ratio IL-8/GAPDH (A, C) and ICAM-1/GAPDH (B,D) between non-infected and infected cells. Results are expressed as mean±SEM of duplicate wells. Representative of 9 independents experiments (A) or 6 (B, C and D) in duplicate are shown.

activation after treatment with miglustat. It should be noted that no correction is observed after 24 h of treatment with miglustat (Fig. 1B). The results obtained in CuFi-1 cells are reported in Fig. 1C. In agreement with IB3-1 cells, no changes of the fluorescent signal are obtained in untreated cells or in cells treated with NB-DGJ. As demonstrated by the increase of the fluorescence after the addition of the cAMP stimulation cocktail plus genistein and by the decrease after the addition of the inhibitor, a correction of F508del-CFTR function is observed after 2, 4 and more evident, 24 h of treatment. Collectively, these results indicate a fast rescue of F508del-CFTR function both in IB3-1 and CuFi-1 CF cells.

In order to evaluate the half maximal effective concentration of miglustat, CFTR-dependent Cl-transport was studied in CuFi-1 cells by iodide efflux. Cells were incubated in the presence of miglustat ranging from 1 nM to 100 μM and iodide efflux was performed as described above. Fig. 2A shows the correction of CFTR function by miglustat in CuFi-1 cells starting from 10 nM, being 76 nM the half maximal effective concentration (Fig. 2B).

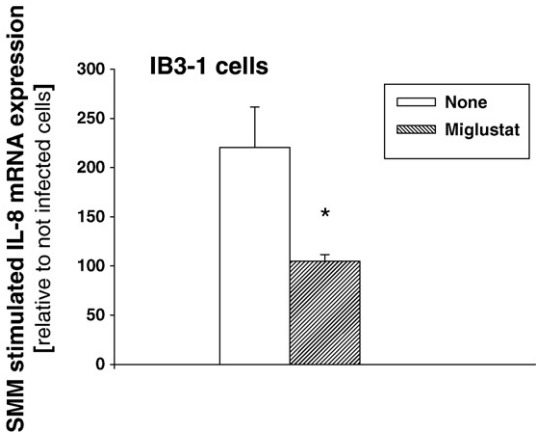


Fig. 4. Miglustat inhibits the inflammatory response to SMM in IB3-1 cells. Cells were incubated for 24 h in the presence of 200 μM miglustat and then stimulated by SMM diluted 1:25 in medium, for 4 h. The IL-8 mRNA induction (relative to not infected cells) is obtained as indicated in the legend of Fig. 3. Results are expressed as mean±SEM of duplicate wells. Representative of 2 independent experiments, in duplicate, is shown.

### 3.2. Effect of miglustat on the expression of inflammatory mediators in IB3-1 cells and CuFi-1 cells stimulated by PAO1

The principal aim of this study was to evaluate the effect of correctors of F508del-CFTR function on the modulation of the expression of pro-inflammatory mediators, induced by *P. aeruginosa* infection in respiratory epithelial cells. IL-8 is an essential component of the host defense system, being released from respiratory epithelial cells in response to bacteria, to regulate transepithelial migration and activation of neutrophils [27]. ICAM-1 is constitutively expressed on the cell surface of a wide variety of cells and it is modulated in response to different inflammatory stimuli, including bacteria [28]. As we have previously demonstrated that overnight incubation of CF bronchial cells with the corrector MPB-07 down-modulates both the mRNA levels and protein expression of IL-8 and ICAM-1, stimulated by PAO1 [8], the effect of miglustat was studied in the same experimental model in IB3-1 and CuFi-1 cells using quantitative real-time RT-PCR as analytical methodology. Miglustat significantly inhibits the PAO1 induced accumulation of both IL-8 ($p<0.0001$) and ICAM-1($p<0.0009$) mRNAs in IB3-1 cells (Fig. 3A and B). Also in CuFi-1 cells miglustat significantly inhibits IL-8 ($p<0.003$) and ICAM-1 ($p<0.03$) mRNA accumulation (Fig. 3C and D). Considering

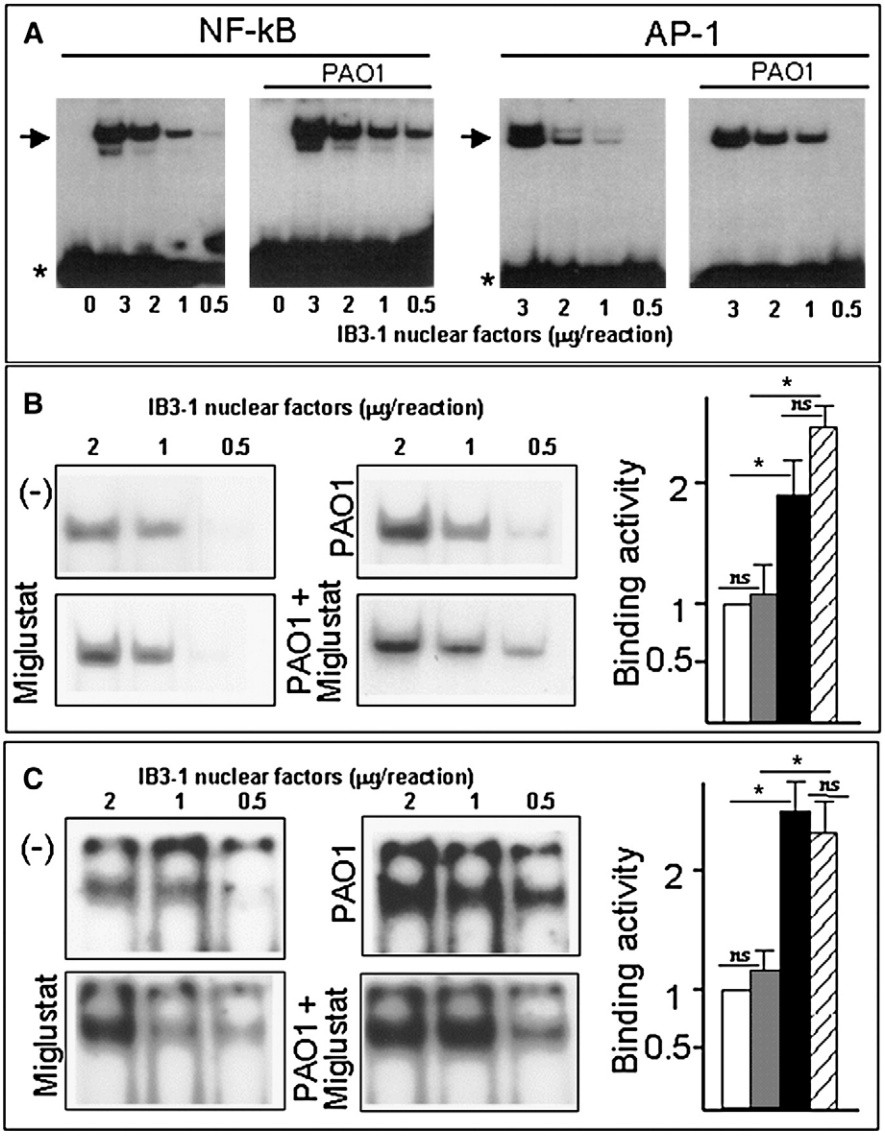


Fig. 5. A. Increase of NF-κB and AP-1 binding activity following PAO1 infection. Nuclear extracts were isolated from uninfected and PAO1 infected cells. Increasing amounts (μg/reactions) of nuclear proteins were mixed with $^{32}$P-labelled NF-κB and AP-1 target oligonucleotides and protein/DNA complexes (arrowed) analysed by gel-shift. Asterisks indicate free $^{32}$P-labelled NF-κB and AP-1 probes. B,C. Miglustat has no major effect on NF-κB and AP-1 overall binding activity. IB3-1 cells were cultured in the absence (upper gels in each panel) or in the presence (lower gels in each panel) of 200 μM miglustat for 24 h and then infected or not with 50 CFU/cell of PAO1 for 2 h, as indicated. Nuclear extracts from these treated cells, as indicated, were isolated and increasing amounts of them (μg/reactions) were mixed with NF-κBNF-*k*B (B) and AP-1 (C) target $^{32}$P-labelled oligodeoxynucleotides and EMSA performed. In the right side of panels B and C quantitative analysis of DNA–protein interactions obtained using 0.5 μg /reaction nuclear factors, performed using the Bio-Rad Gel-Doc densitometry system is shown. The results reported represent binding activities relative to control protein extracts isolated from untreated control cells (−) (average±SD from three independent experiments). Open bars=no miglustat, no PAO1; grey bars: miglustat; black bars: PAO1; dashed bars: PAO1+miglustat.

that chronic lung infection in the airways of CF patients is sustained in the advanced stages by non-motile *P. aeruginosa* growing in biofilms, we tested the effect of a potent pro-inflammatory stimulus deriving from the bronchial lumina of CF patients undergoing lung transplantation [18–19]. Pooled supernatants of mucopurulent material (SMM), obtained from human CF lungs infected with *P. aeruginosa* and *S. aureus* [18–19], were tested in IB3-1 cells upon treatment with miglustat. The significant inhibition of the expression of IL-8 stimulated by SMM, shown in Fig. 4, strengthens the potential anti-inflammatory effect of miglustat in reducing the *P. aeruginosa*-dependent transcription of two critical genes involved in leukocyte chemotaxis, such as IL-8 and ICAM-1.

### 3.3. DNA-binding activity of NF-κB and AP-1 by P. aeruginosa in cells treated with miglustat

The inhibitory effect observed on IL-8 and ICAM-1 transcription in CF airway epithelial cell lines opens the possibility that miglustat could intervene in the signalling cascade between the receptors recognized by *P. aeruginosa* and the transcription of IL-8 and ICAM-1, besides correcting F508del-CFTR. Attention was therefore drawn on the transcription factors NF-κBNF-*k*B and AP-1, which play a central role in the immune response to *P. aeruginosa* infection in respiratory epithelial cells [29] and have already demonstrated to be activated in IB3-1 and CuFi-1 cells upon infection with PAO1 [8]; accordingly, putative target consensus sequences of these two transcription factors have been found within the promoter sequences of both IL-8 and ICAM-1 genes (TESS software analysis) (www.cbill.upenn.edu/cgi_bin/tess/tess). In order to determine whether miglustat treatment modulates the overall NF-κBNF-*k*B and AP-1 binding activity the experiment shown in Fig. 5 was performed. IB3-1 cells were cultured in the absence or in the presence of miglustat and then infected or not with PAO1 for 2 h. Nuclear extracts were isolated, increasing amounts of them were mixed with NF-κBNF-*k*B and AP-1 target $^{32}$P-labelled oligodeoxynucleotides and EMSA was performed. The results clearly allow these conclusions: (a) both NF-κBNF-*k*B and AP-1 binding activity significantly increases following *P. aeruginosa* infection of IB3-1 cells ($p<0.05$) (see data reported in Fig. 5A) and (b) no significant major alteration of this binding activity occurs in both uninfected or PAO1 infected cells treated with miglustat (see data reported in Fig. 5, B and C). These results were consistently obtained in three independent experiments. Therefore miglustat has no major effect on overall binding activity of the transcription factors NF-κBNF-*k*B and AP-1.

### 3.4. Effect of NB-DGJ on PAO1 stimulated IL-8 MRNA expression in IB3-1, CuFi-1 and NuLi-1 cells

To verify whether the anti-inflammatory effect of miglustat observed in IB3-1 and CuFi-1 cells might be related to the correction of F508del-CFTR function, cells were also treated with NB-DGJ, which does not restore CFTR-dependent Cl- transport (Fig. 1). As shown in Fig. 6A and B, also NB-DGJ significantly reduces the expression of IL-8 mRNA stimulated by PAO1 in IB3-1 ($p<0.003$) and CuFi-1 cells ($p<0.04$). These experiments were extended to NuLi-1 cells, which express wild type CFTR protein. As shown in Fig. 6C miglustat significantly decreases the amount of IL-8 mRNA also in NuLi-1 cells ($p<0.02$). Moreover, NB-DGJ produces a significant inhibition of the transcription of IL-8 stimulated by PAO1 ($p<0.04$) although to a lower extent of that obtained with miglustat ($p<0.03$). On the whole, these results indicate that the anti-

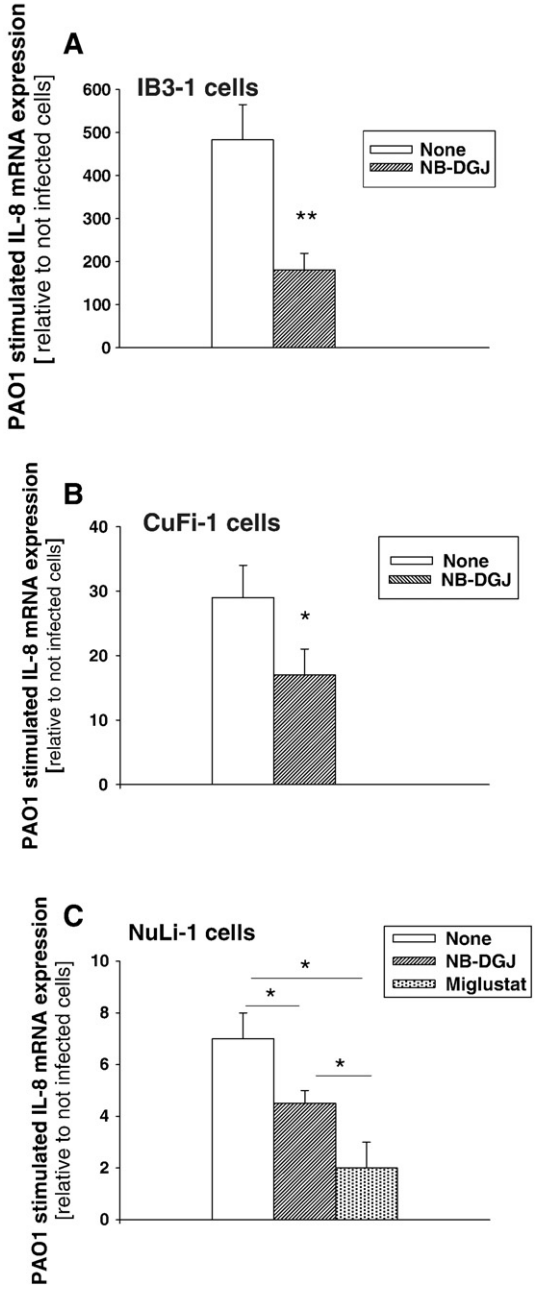


Fig. 6. Both miglustat and NB-DGJ reduce the PAO1 stimulated IL-8 mRNA expression in CF and non-CF bronchial cells. Cells were incubated with miglustat or NB-DGJ (200 μM) for 24 h as described in Fig. 3. The mRNA induction (relative to non-infected cells) is obtained by comparing the ratio IL-8/GAPDH between non-infected and infected cells. Results are expressed as mean ± SEM of duplicate wells. Representative of 3(A), 4(B) and 5(C) independent experiments in duplicate are shown.

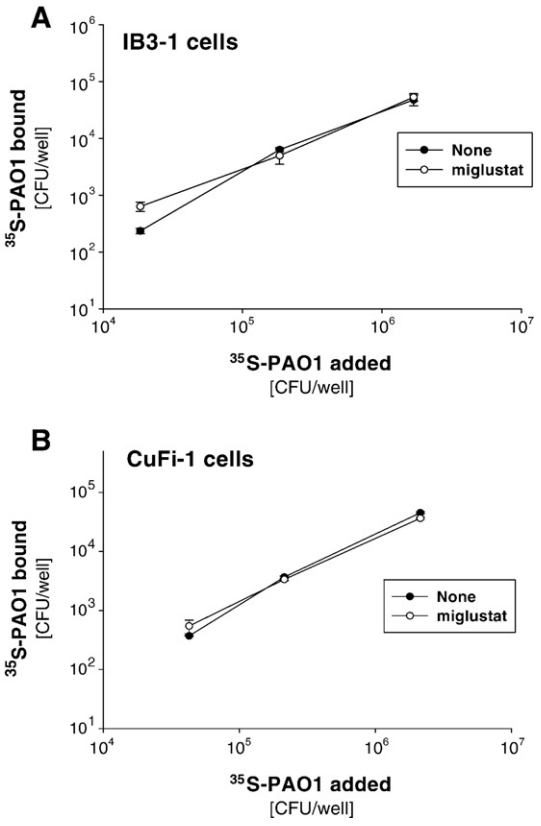


Fig. 7. Miglustat does not reduce the binding of PAO1 to IB3-1 and CuFi-1 cells. 500,000 IB3-1 (A) or CuFi-1 (B) cells on Petri dishes, in duplicate, were treated for 24 h with 200 μM miglustat and then washed with PBS before performing adhesion experiments. Different amounts of $^{35}$S-PAO1, expressed as CFU/well, were added to the wells and incubated as described in Materials and methods section. Data reported in the figure are the specific binding calculated by subtracting counts obtained in the presence of 100-fold excess of non-labelled PAO1 and are expressed as CFU/well. Representative of two independent experiments are reported in the figure.

inflammatory action of miglustat is independent of the correction of F508del-CFTR in IB3-1 and CuFi-1 cells.

### 3.5. Adherence of PAO1 to IB3-1 and CuFi-1 cells treated with miglustat

Many pathogenic microorganisms use glycoconiugate receptors to establish contact with the host tissues [30] and pharmacological agents that inhibit the biosynthesis of these receptors may have a major impact on the pathogenesis of infection. Miglustat was shown to decrease the GSL content, to inhibit the P-fimbriated bacterial attachment and, as a consequence, to impair the mucosal inflammatory response in mice [31]. *P. aeruginosa* flagellin can interact with both Toll-like receptor 5 (TLR5) and the cell surface glycolipid, asialoGM1, to activate the innate immune response [29]. Therefore the anti-inflammatory effect of miglustat observed in IB3-1 and CuFi-1 cells could reflect the reduced expression of the glycolipid receptors for PAO1. To evaluate this possibility the adherence of metabolically labelled [$^{35}$S] methionine-PAO1 was measured in IB3-1 and CuFi-1 cells, treated or not with miglustat. Fig. 7A and B show a dose dependent increase of the PAO1 binding to the cells and no significant differences due to the treatment with miglustat. These results indicate that incubation with miglustat for 24 h does not affect the adherence of PAO1 to IB3-1 and CuFi-1 cells, suggesting that the huge inhibition of *P. aeruginosa*-dependent IL-8 and ICAM-1 transcription after treatment with miglustat is not dependent upon reduction of bacterial-host cell interactions.

### 3.6. Effect of miglustat and NB-DGJ on the expression of IL-8 mRNA stimulated by PAO1, TNF alpha or IL-1 beta in IB3-1 and CuFi-1 cells

The results shown so far indicate that miglustat and NB-DGJ have an anti-inflammatory effect in bronchial epithelial cells, suggesting that they could inhibit the transmembrane signalling between the receptors for pathogens and the transcription of the inflammatory genes. To obtain preliminary insights on which transduction pathway is inhibited by miglustat and NB-DGJ, the expression of IL-8 mRNA stimulated by either PAO1 or the pro-inflammatory cytokines TNF alpha and IL-1 beta, was studied both in IB3-1 and in CuFi-1 cells. As depicted in Fig. 8A, miglustat and NB-DGJ significantly inhibit the expression of

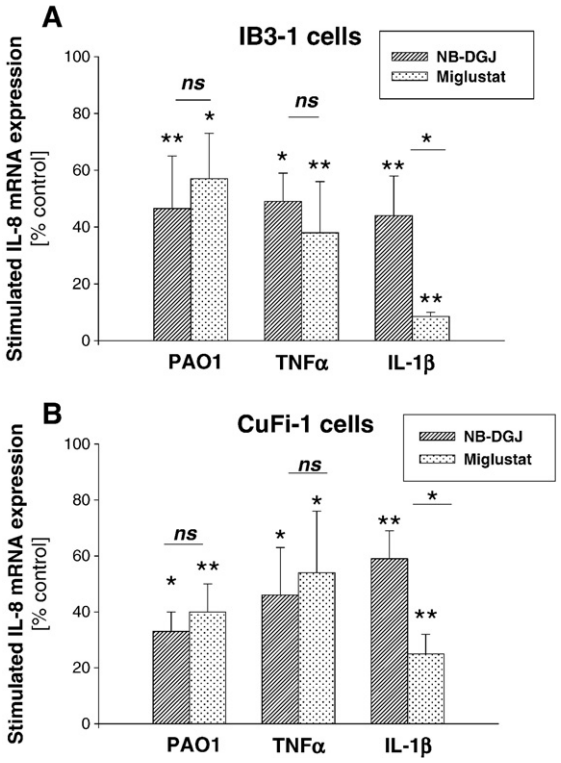


Fig. 8. Miglustat and NB-DGJ reduce the expression of IL-8 mRNA stimulated by PAO1, or TNF alpha or IL-1 beta in IB3-1 and CuFi-1 cell lines. Cells were incubated for 24 h with miglustat or NB-DGJ (200 μM) and then infected with PAO1 as above described or stimulated with TNF alpha (10 ng/ml) or IL-1 beta (50 ng/ml) for 4 h. Stimulated IL-8 mRNA expression was calculated as indicated in the legend of Fig. 3 and it is expressed as % of untreated cells. A. In untreated IB3-1 cells the stimulated IL-8 mRNA expression was 407±80 by PAO1, 51±6 by TNF alpha and 148±15 by IL-1 beta ($n$=4). B. In untreated CuFi-1 cells the stimulated IL-8 mRNA expression was 52±13 by PAO1, 6±1 by TNF alpha and 9±1 by IL-1 beta ($n$=4). Representative of 2 independent experiments in duplicate are shown.

IL-8 mRNA not only after stimulation by PAO1 but also by TNF alpha or IL-1 beta , in IB3-1 cells. Fig. 8B confirms these findings also in CuFi-1 cells. Interestingly, miglustat produces a significantly stronger reduction of IL-1 beta elicited IL-8 mRNA expression than that given by NB-DGJ, in IB3-1 and CuFi-1 cells, whereas no differences are observed both upon infection with PAO1 and induction by TNF alpha. Therefore, miglustat and NB-DGJ inhibit the pro-inflammatory signalling downstream the receptors for PAO1 and for these pro-inflammatory cytokines.

## 4. Conclusions

We recently demonstrated that miglustat, an inhibitor of the ER alpha-1,2-glucosidase, restores F508del-CFTR function in human nasal epithelial JME/CF15 cells by preventing the interaction between F508del-CFTR and calnexin [17]. Folding and trafficking of proteins to their correct cellular location depend on molecular chaperones which can have different effects on each particular substrate and particular cell type [32]. As a consequence, the effectiveness of small correctors of protein trafficking defect may depend on the cellular environment and may be cell type specific. The rescue of CFTR function by miglustat has been observed in different cells, such as nasal JME/CF15, tracheal gland serous CFKM4, pancreatic duct CFPAC-1 cell lines, intestinal cells of F508del-CFTR mice [17]. Here we report a correcting effect in the CF bronchial IB3-1 and CuFi-1 cells (Figs. 1 and 2), although we observed a lack of rescue of CFTR function in IB3-1 cells treated with miglustat for 24 h (Fig. 1B). Experience from the newly discovered F508del-CFTR correctors starts indicating that the potency of action can be quite different, or sometimes even absent, when tested in different cell models [32]. In respect to miglustat, it should be recalled that it corrects F508del-CFTR by inhibiting the α-glucosidase of the ER [17], an effect which can vary upon the cells line model and the culture conditions. Moreover, CuFi-1 cells are homozygous for the F508del-CFTR mutation whereas IB3-1 cells are compound heterozygous for the F508del-CFTR mutation (genotype F508del/W1282X). As the amount of F508del proteins in IB3-1 cells may be quite different compared to that of CuFi-1 cells this may also contribute to the variability of the correcting effect, which anyway requires further investigation.

In this study we have shown that miglustat: i) is a corrector of F508del-CFTR function in CF bronchial IB3-1 and CuFi-1 cells; ii) has an anti-inflammatory effect in bronchial cells independently of the correction of F508del-CFTR; iii) has no effect on NF-κB and AP1 overall binding activity; iv) inhibits the pro-inflammatory signalling downstream both the receptors for pathogens and key pro-inflammatory cytokines.

Therefore, besides confirming the correction of F508del-CFTR function, we have demonstrated that miglustat reduces the inflammatory response elicited by *P. aeruginosa* or SMM in CF bronchial IB3-1 and CuFi-1 cells (Figs. 3 and 4), supporting the hypothesis that the pharmacological modulation of CFTR defect may reduce the excessive lung inflammatory response in CF cells, consistent with previous findings showing an anti-inflammatory effect of the corrector MPB-07 [8]. However, also the galactose analog of miglustat, NB-DGJ, which is not a corrector of F508del-CFTR function, reduces the inflammatory response in CF cells and both the compounds have an anti-inflammatory effect in non-CF bronchial NuLi-1 cells (Fig. 6). Therefore, although miglustat rescues functional F508del-CFTR in CF bronchial cells, it affects the inflammatory response to *P. aeruginosa* through a mechanism which, at least partly, is independent of the correction of F508del-CFTR function.

How this occurs is a matter of speculation. Both analogues, miglustat and NB-DGJ, are inhibitors of the first step in GSL biosynthesis which is the transfer of glucose to ceramide by the ceramide-specific glucosyl-transferase (CerGlcT) [30], whereas miglustat, but not NB-DGJ, also inhibits ER alpha-glucosidase [16]. The fact that correction of F508del-CFTR function is obtained in cells treated with miglustat but not with NB-DGJ, supports that the correcting effect is specifically related to ER alpha-glucosidase inhibition, thus preventing the interaction of F508del-CFTR with calnexin [17]. On the other hand the anti-inflammatory effect observed in cells treated with either miglustat or NB-DGJ suggests a mechanism involving the inhibition of CerGlcT, an activity shared by both compounds. Nevertheless, depending on cell type and pro-inflammatory stimulus, miglustat seems to be more effective than NB-DGJ (Figs. 3C and 8). Thus, also ER alpha-glucosidase could play a role in pro-inflammatory signaling. CerGlcT is crucial for the synthesis of GSL and it is the target for treating GSL lysosomal storage disease in the therapeutic approach termed substrate reduction therapy [16]. Gaucher disease is the most frequently occurring GSL storage disease, characterized by accumulation of glucosylceramide causing heterogeneous clinical features, among which sustained inflammatory reaction associated with elevated levels of pro-inflammatory cytokines [33]. It has been suggested that GSL may act as signalling molecule to dysregulate the immune system in Gaucher disease [34]. Interestingly, mouse models of GSL storage diseases treated by substrate reduction therapy with miglustat, display a marked reduction in systemic inflammation [35], strengthening the link between the accumulation of GLS and the extent of the inflammatory response. Very importantly, the accumulation of the sphingolipid ceramide has been recently identified as one of the key regulators of inflammation and infection in CF airways [36]. We can thus argue that miglustat reduces the inflammatory response by interfering with ceramide signalling. The transcription factors NF-κBNF-*k*B and AP-1 play a central role in the immune response to *P. aeruginosa* infection in respiratory epithelial cells [29]. Interestingly, an alternative pathway, independent of NF-κB, operating through prostaglandin E2 receptor, has been demonstrated to regulate IL-8 secretion in IB3-1 cells [37]. A likely inhibition of the overall binding activity of NF-κBNF-*k*B and AP-1 by miglustat has been ruled out from the data reported in Fig. 5, suggesting that it could interfere with other pathways in signal transduction. Future studies on the potential effect of miglustat on the complex array of transcription factors regulating IL-8, ICAM-1 and other genes of the innate immune response are needed to ascertain this point.

The anti-inflammatory effect of miglustat and NB-DGJ could reflect the reduced expression of the glycolipid receptors

for *P. aeruginosa* due to a decrease in the cellular GSL content as a consequence of the inhibition of CerGlcT activity. Here we show that miglustat does not reduce the adherence of *P. aeruginosa* to the IB3-1 cells (Fig. 7), thus making unlikely that the anti-inflammatory effect of miglustat is due to interference with the receptors for *P. aeruginosa*. Moreover, since miglustat strongly reduces the pro-inflammatory signals elicited also by TNF alpha and IL-1 beta (Fig. 8), these compounds could possibly modulate directly or indirectly the activity of some of the different kinases or adapters downstream the receptors for pathogens, in common with TNF alpha and IL-1 beta receptors through a mechanism that needs further investigation. From the practical point of view, the results presented in this paper show that miglustat has two therapeutically relevant effects in CF cells: it rescues the F508del-CFTR function and reduces the inflammatory response to *P. aeruginosa*. Interestingly, both correction of the cystic fibrosis defect and inhibition of anti-inflammatory responses are of great importance in the therapy of CF patients. Accordingly, a drug displaying these two effects deserves great attention. Furthermore, the effects of miglustat on human subjects are well known. In fact, miglustat is an orally bioavailable drug approved in Europe and USA for the treatment of Gaucher disease and other GSL storage diseases. Human studies show that it is well tolerated at 100 or 300 mg once or three times daily [38]. The pharmacokinetic properties of miglustat, studied at the dosage used in the treatment of type 1 Gaucher's disease [15] indicate that the drug is rapidly absorbed after oral administration, with a peak plasma concentration of 0.86 μM/mL at 2.5 h. In clinical trials, for a dosage of 100 mg three times daily, steady-state plasma concentration of 1.5 μM/mL was attained within 4–6 weeks [15,39]. Although studies in murine models indicate that lung tissue concentrations reach equimolar levels with those in plasma [40], to the best of our knowledge no data on human lung tissue concentrations have been studied after oral treatment. Therefore, further investigation in animal models will be necessary to start understanding the effective anti-inflammatory concentration *in vivo*. Moreover, analysis of markers of inflammation in CF patients undergoing clinical trials with oral miglustat as a CFTR corrector (http://clinicaltrials.gov/) will likely help elucidating this important issue, which is difficult to predict simply from *in vitro* studies.

All this considered, miglustat may represent a promising candidate for the pharmacotherapy of CF and an interesting research tool to investigate the immune response of airway cells.

## 5. Conflict of interest statement

All authors disclose any financial and personal relationships with other people or organizations that could inappropriately influence this work.

## Acknowledgements

We are grateful to A. Tamanini for the helpful discussions, A. Prince for the *P. aeruginosa* laboratory strain PAO1, M.G. Giri for the statistical analysis, F. Quiri for excellent technical assistance, In Vitro Model and Cell Culture Care of the University of Iowa for providing NuLi-1 and CuFi-1 cells and A.S. Verkman for $CFTR_{inh}$-172.

This research was supported by the Italian Cystic Fibrosis Research Foundation (grant FFC # 16/2006) with the contribution of "Associazione Veneta per la lotta alla Fibrosi Cistica", Fondazione Cariverona — Bando 2005 — Malattie rare e della povertà (to GC) and by Vaincre la Mucoviscidose (to CN and FB).

## References

[1] Govan JRW, Deretic V. Microbial pathogenesis in cystic fibrosis: mucoid *Pseudomonas aeruginosa* and *Burkholderia cepacia*. Microbiol Rev 1996;60:539–74.
[2] Bonfield TL, Panuska JR, Konstan MW, Hilliard JB, Hilliard JB, Ghnaim H, et al. Inflammatory cytokines in cystic fibrosis lungs. Am J Respir Crit Care Med 1995;152:2111–8.
[3] Dakin CJ, Numa AH, Wang H, Morton JR, Vertzyas CC, Henry RL. Inflammation, infection and pulmonary function in infants and young children with cystic fibrosis. Am J Respir Crit Care Med 2002;165:904–10.
[4] Matsui H, Grubb BR, Tarran R, Randell SH, Gatzy JT, Davis CW, et al. Evidence for periciliary liquid layer depletion, not abnormal ion composition, in the pathogenesis of cystic fibrosis airway disease. Cell 1998;95:1005–15.
[5] Machen TE. Innate immune response in CF airway epithelia: hyperinflammatory? Am J Physiol Cell Physiol 2006;291:C218–30.
[6] Ma T, Vetrivel L, Yang H, Pedemonte N, Zegarra-Moran O, Galietta LJV, et al. High-affinity activators of cystic fibrosis transmembrane conductance regulator (CFTR) chloride conductance identified by throughput screening. J Biol Chem 2002;277:37235–41.
[7] Perez A, Issler AC, Cotton CU, Kelley TJ, Verkman AS, Davis PB. CFTR inhibition mimics the cystic fibrosis inflammatory profile. Am J Physiol Lung Cell Mol Physiol 2007;292:L383–95.
[8] Dechecchi MC, Nicolis E, Bezzerri V, Vella A, Colombatti M, Assael BM, et al. MPB-07 reduces the inflammatory response to *Pseudomonas aeruginosa* in cystic fibrosis bronchial cells. Am J Respir Cell Mol Biol 2007;36:615–24.
[9] Pedemonte N, Diena T, Caci E, Nieddu E, Mazzei M, Ravazzolo R, et al. Antihypertensive 1,4-dihydropyridines as correctors of the cystic fibrosis transmembrane conductance regulator channel gating defect caused by cystic fibrosis mutations. Mol Pharmacol 2005;68:1736–46.
[10] Van Goor F, Straley KS, Cao D, Gonzàles J, Hadida S, Hazlewood, et al. Rescue of ΔF508 CFTR trafficking and gating in human cystic fibrosis airway primary cultures by small molecules. Am J Physiol Lung Cell Mol Physiol 2006;290:L1117–30.
[11] Pedemonte N, Lukacs GL, Du K, Caci E, Zegarra-Moran O, Galietta LJV, et al. Small-molecule correctors of defective DF508-CFTR cellular processing identified by high-throughput screening. J Clin Invest 2005;115:2564–71.
[12] Wang Y, Bartlett MC, Loo TW, Clarke DM. Specific rescue of the cystic fibrosis transmembrane conductance regulator processing mutants using pharmacological chaperones. Mol Pharmacol 2006;70:297–302.
[13] Asano N. Glycosidase inhibitors: update and perspectives on practical use. Glycobiology 2003;13:93R–104R.
[14] Platt FM, Neises GR, Dwek RA, Butters TD. *N*-butyldeoxynojirimycin is a novel inhibitor of glycolipid biosynthesis. J Biol Chem 1994;269:8362–5.
[15] Cox T, Lachmann R, Hollak C, Aerts J, van Weely S, Hrebicek M, et al. Novel oral treatment of Gaucher's disease with *N*-butyldeoxynojirimycin (OGT 918) to decrease substrate biosynthesis. Lancet 2000;355:1481–5.
[16] Butters TD, Dwek RA, Platt FM. Imino sugar inhibitors for treating the lysosomal Glycoshingolipidoses. Glycobiology 2005;15:43R–52R.
[17] Norez C, Noel S, Wilke M, Bijvelds M, Jorna H, Melin P, et al. Rescue of functional delF508-CFTR channels in cystic fibrosis epithelial cells by the α-glucosidase inhibitor miglustat. FEBS Lett 2006;580:2081–6.

[18] Ribeiro CMP, Paradiso AM, Carew MA, Shears SB, Boucher RC. Cystic fibrosis airway epithelial $Ca^{2+}i$ signaling. The mechanism for the larger agonist-mediated $Ca^{2+}i$ signals in human cystic fibrosis airway epithelia. J Biol Chem 2005;280:10202–9.
[19] Ribeiro CMP, Paradiso AM, Schwab U, Perez-Vilar J, Jones L, O'Neal W, et al. Chronic airway infection/inflammation induces a $Ca^{2+}{}_i$-dependent hyperinflammatory response in human cystic fibrosis airway epithelia. J Biol Chem 2005;280:17798–806.
[20] Zeitlin PL, Lu L, Rhim J, Cutting G, Stetten G, Kieffer KA, et al. A cystic fibrosis bronchial epithelial cell line: immortalization by adeno-12-SV40 infection. Am J Respir Cell Mol Biol 1991;4:313–9.
[21] Zabner J, Karp P, Seiler M, Phillips Sl, Mitchell CJ, Saavedra M, et al. Development of cystic fibrosis and non-cystic fibrosis airway cell lines. Am J Physiol Lung Cell Mol Physiol 2003;284:L844–54.
[22] Ma T, Thiagarajah JR, Yang H, Sonawane ND, Folli C, Galietta LJV, et al. Thiazolidinone CFTR inhibitor identified by high-throughput screening blocks cholera-toxin induced intestinal fluid secretion. J Clin Invest 2002;110:1651–8.
[23] Norez C, Antigny F, Becq F, Vandebrouck C. Maintaining low $Ca^{2+}$ level in the endoplasmic reticulum restores abnormal endogenous F508del-CFTR trafficking in airway epithelial cells. Traffic 2006;7:562–73.
[24] Andrews NC, Faller DV. A rapid micropreparation technique for extraction of DNA-binding proteins from limiting numbers of mammalian cells. Nucleic Acids Res 1991;19:2499.
[25] Borgatti M, Lampronti I, Romanelli A, Pedone C, Saviano M, Bianchi N, et al. Transcription factor decoy molecules based on a peptide nucleic acid (PNA)–DNA chimera mimicking Sp1 binding sites. J BiolChem 2003;278:7500–9.
[26] Saiman L, Prince A. *Pseudomonas aeruginosa* pili bind to asialoGM1 which is increased on the surface of cystic fibrosis epithelial cells. J Clin Invest 1993;92:1875–1880.25.
[27] Diamond G, Legarda D, Ryan LK. The innate immune response of the respiratory epithelium. Immunol Rev 2000;173:27–38.
[28] Roebuck KA, Finnegan A. Regulation of intercellular adhesion molecule-1 (CD54) gene expression. J Leukoc Biol 1999;66:876–88.
[29] McNamara N, Gallup M, Sucher A, Maltseva I, McKemy D, Bausbaum C. AsialoG and TLR5 cooperate in flagellin-induced nucleotide signalling to activate Erk1/2. Am J Respir Cell Mol Biol 2006;34:653–60.
[30] Dwek RA, Butters TD, Platt FM, Zitzmann N. Targeting glycosilation as a therapeutic approach. Nat Rev Drug Discov 2002;1:65–75.
[31] Svensson M, Frendeus B, Butters T, Platt F, Dwek R, Svanborg C. Glycolipid depletion in antimicrobial therapy. Mol Microbiol 2003;47:453–61.
[32] Amaral M. Therapy through chaperones: sense or antisense? Cystic fibrosis as a model disease. J Inherit Metab Dis 2006;29:477–87.
[33] Beutler E, Grabowski GA. Gaucher disease. In: Scriver CR, Beaudet AL, Sly WS, Valle D, editors. The metabolic and molecular bases of inherited disease. New York, New York, USA: McGraw-Hill; 2001. p. 3635–68.
[34] Mizukami H, Mi Y, Wada R, Kono M, Yamashita T, Liu Y, et al. Systemic inflammation in glucocerebrosidase-deficient mice with minimal glucosylceramide storage. J Clin Invest 2002;109:1215–21.
[35] Jeyakumar M, Thomas R, Elliot-Smith E, van der Spoel AC, d'Azzo A, Hugh Perry V, et al. Central nervous system inflammation is a hallmark of pathogenesis in mouse models of GM1 and GM2 gangliosidosis. Brain 2003;126:974–87.
[36] Teichgraber V, et al. Ceramide accumulation mediates inflammation, cell death and infection susceptibility in cystic fibrosis. Nat Med 2008;14:382–91.
[37] Vij N, Amoako MO, Mazur S, Zeitlin PL. CHOP transcription factor mediates IL-8 signaling in cystic fibrosis bronchial epithelial cells. Am J Respir Cell Mol Biol 2008;38:176–84.
[38] Cox TM, et al. Advisory Council to the European Working Group on Gaucher disease. J Inherit Metab Dis 2003;26:513–26.
[39] McCormack PL, Goa KL. Miglustat. Drugs 2003;63:2427–34.
[40] Treiber A, Morand O, Clozel M. The pharmacokinetics and tissue distribution of the glucosylceramide synthase inhibitor miglustat in the rat. Xenobiotica 2007;37:298–314.